(A266C/I1115C), NBD1/ICL4 (A481C/S909C) and NBD1/NBD2 (A431C/A1074C). All the mutants showed modulation of crosslinking with change in protein conformation from APO to ADP-trapped. In the mutant T173C/T816C, the transmission of signal from the ATP-binding sites to TM3 and TM9 may occur via intracellular loops ICL1 and ICL3. This is highlighted by the fact that substitution of conserved residues D164 and D805 with Cys in ICL1 and ICL3, respectively reduced the basal ATPase activity and was unaffected by addition of inhibitors such as tariquidar and cyclosporine A. In addition, Cys sites in the Walker A domain of both NBD's cross linked in apo conformation to 20-25 Å long crosslinkers. This suggests that the ATP sites are much closer than predicted by the homology model of human Pgp (36 Å) based on the mouse Pgp structure. In aggregate, the broad distance measurements from PDS and disulfide crosslinking suggest high degree of flexibility in Pgp.

3084-Plat
Ouabain Binding Site in a Functioning Na+/K+-ATPase
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The Na+/K+ ATPase is an almost ubiquitous integral membrane protein within the animal kingdom. It is also the selective target for cardiotonic derivatives, widely prescribed inhibitors for patients with heart failure. Functional studies revealed that ouabain-sensitive residues were distributed widely throughout the primary sequence of the α subunit, which spans ten times across the cell membrane and contains all the necessary components for ion transport: the ion permeation pathway, the phosphorylation site and the ATP binding domain protein. Recently, structural work has revealed that ouabain binds at the external end of the ion permeation pathway. To elucidate the ouabain binding site in a functioning Na+/K+ ATPase we use a spectroscopic approach that estimates distances between a fluorescent ouabain and a lanthanide binding tag (LBT). We introduced LBTs at five different positions in the Na+/K+ ATPase sequence. These five normally functional LBT-Na+/K+ ATPase constructs were expressed in the cell membrane of Xenopus laevis oocytes, operating under physiological internal and external ion conditions. The spectroscopic data suggest two mutually exclusive distances between the LBT and the fluorescent ouabain. From the estimated distances and using homology models of the LBT-Na+/K+ ATPase constructs, approximate ouabain positions could be determined. Our results suggest that ouabain binds at two sites along the ion permeation pathway of the Na+/K+ ATPase. The external site (low apparent affinity) occupies the same region as previous structural findings. The high apparent affinity site is, however, slightly deeper towards the intracellular end of the protein. Interestingly, in both cases the lactone ring faces outward. We propose a sequential ouabain binding mechanism that is consistent with all functional and structural studies. This work was supported by NIH grants R01-GM062342, R01-GM030376 and U54-GM087519, and the Intramural Research Program of the National Institutes of Health, NINDS.

3085-Plat
Study of Nucleotide Binding to the Uncoupling Protein 1 using Atomic Force Microscopy
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A tight proton transport regulation in the inner mitochondrial membrane is crucial for physiological processes such as ATP synthesis or non-shivering thermogenesis in the presence of uncoupling protein-1 (UCP1). Several hypotheses explaining the inhibitory effect of ATP on UCP were proposed, however, structural details are missing so far. Here we use the topographic and recognition (TREC) mode of an atomic force microscope to visualize UCP1 re-constituted into lipid bilayers (Beck, 2006) and to analyze the ATP-protein interaction on the single molecule level (Wildling, 2011). The comparison of recognition patterns obtained with anti-UCP antibody and ATP led to the conclusion that the ATP binding site can be accessed from both membrane sides. Using cantilever tips with different crosslinker lengths, we determined the location of the nucleotide binding site inside the membrane with 1 Å precision. In the absence of the crystallographic structure for UCP1, these data provide valuable insight into the nucleotide binding mechanism.
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Platform: Protein Structure

3086-Plat
NMR Structure of VarI, a Novel Bioactive Peptide Toxin from Terebrid Marine Snails
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Peptide toxins display great specificity in both targeting and function, and are a viable resource for enriching the available arsenal of pharmaceutical compounds. For example Ziconotide (Prialt), a peptide toxin derived from cone snails (conotoxin), is the first FDA approved conotoxin drug used to treat chronic pain in cancer and HIV patients by targeting voltage dependent Ca2+ channels. Cone snail toxins have long been identified as effective compounds for characterizing ion channels and receptors. However, the Terebridae, a less studied, related venomous snail family, are also a pharmacologically relevant source of toxins. Similar to cone snails, genomic and proteomic analyses of terebrid venom reveal a vibrant diversity of peptide toxins (teretoxins). Here, we present the first structural elucidation of a teretoxin, VarI. VarI was chemically synthesized, oxidatively refolded, and structurally characterized by nuclear magnetic resonance (NMR) spectroscopy. Standard homonuclear NMR methods were used in the structure determination, and the final bundle of lowest energy structures revealed an unusual pattern of disulfide bond connectivities potentially unique to teretoxins. The NMR structure of Var1 will assist in understanding the toxin's pharmacological properties, and is being used to guide the search for functional receptors of VarI.

3087-Plat
Coordination of Hydrogen Peroxide by a Copper Center in Peptidylglycine Alpha-Hydroxylating Monooxygenase (PHM). Structural and Computational Study
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Many bioactive peptides, such as hormones and growth factors require amidation of the C-terminus for their full biological activity. The enzyme peptidylglycine α-hydroxylating monooxygenase (PHM) carries out the first step of the amidation process, the hydroxylation of peptidylglycine substrates at the Cα position of the terminal glycine. Two non-equivalent copper sites in PHM (Cu_H and Cu_M) play distinct roles in the reaction cycle: Cu_M serves as an oxygen activation and hydrogen abstraction site, while Cu_H is involved in electron transfer. The proposed mechanism suggests that dioxygen is activated through a two-electron reduction, where each of the copper centers provides a single electron. However, there is an ambiguity regarding the characteristics of the reduced oxygen species in the PHM reaction and the identity of the reactive intermediate. To further investigate the nature of the key intermediates in the PHM cycle we determined the structure of the oxidized form of PHM complexed with hydrogen peroxide. In this 1.95 Å resolution structure, the (hydro)peroxide ligand coordinates solely to Cu_M in a slightly asymmetric side-on mode. The copper-oxygen distances are 1.9 and 2.1 Å and the O-O bond 1.5 Å. The interatomic O-O distance is characteristic of peroxide/hydroperoxide species, and is significantly longer than the distance typically observed in the superoxide molecule. In addition to the x-ray diffraction studies, we performed DFT calculations using the first coordination sphere of the Cu_M active site as a model system. Comparison of the relative energies associated with side-on Cu(II)-O_2^{2-} species and its resonant, end-on structure Cu(I)-$O_2^{\bullet-}$ suggests that these two intermediates are significantly populated within the protein environment; this observation has important mechanistic implications.